

# The Autologous Mixed Epidermal Cell-T Lymphocyte Reaction Is Elevated in Psoriasis: A Crucial Role for Epidermal HLA-DR<sup>+</sup>/CD1a<sup>-</sup> Antigen-Presenting Cells

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The objective of this study was to determine whether epidermal cells (EC) from psoriasis lesions and uninvolved skin could stimulate autologous T lymphocytes in the in vitro autologous mixed epidermal cell-T lymphocyte reaction (autologous MECLR). The functional role of antigen-presenting cell (APC) subsets was concurrently determined in this reaction. Mononuclear cells and purified T lymphocytes from peripheral blood of psoriasis patients showed a clear proliferative response to autologous unpurified epidermal cells from involved as well as uninvolved skin. The autologous mixed leukocyte reaction (MLR) was not elevated in psoriasis patients. In healthy controls and contact allergy patients, T-lymphocyte proliferation was not observed either in the autologous MECLR or in the autologous MLR. The level of proliferation in the autologous MECLR from psoriasis patients correlated to the number of epidermal cells that were added. To exclude the possibility that the observed proliferation in the autologous MECLR in psoriasis was due to the presence of epidermal T lymphocytes that were being

stimulated and expanded in vitro, the stimulator EC were gamma irradiated (30 Gy) in some experiments. Preincubation of EC with cyclosporin A (CsA) significantly inhibited the autologous MECLR. The CsA-induced inhibition could be neutralized by the addition of fresh untreated EC to these cultures. This indicated that one of the modes of action of CsA in resolving psoriasis is, as some investigators have already shown, via inhibition of epidermal accessory cell function. In the autologous MECLR, APC from psoriasis skin could initiate this reaction, whereas APC from peripheral blood could not. This occurred in an MHC class II restricted fashion. Depletion experiments showed that Langerhans cells (HLA-DR<sup>+</sup>/CD1a<sup>+</sup>) were not the principal stimulators of autologous T lymphocytes in the MECLR. These results indicated that mainly HLA-DR<sup>+</sup>/CD1a<sup>-</sup> epidermal cells from psoriasis patients could stimulate autologous peripheral blood T lymphocytes in an MHC class II-restricted fashion. *J Invest Dermatol* 96:880-887, 1991

**T**he etiology of psoriasis is still unclear [1-5]. Some early studies led to the assumption that psoriasis was a genetically determined primary keratinocyte disease with the net result of increased proliferation [3]. Keratinocytes have indeed been observed to produce, in vivo and in vitro, a wide array of inflammatory mediators and cytokines including those capable of autocrine stimulation of keratinocyte growth [4-9]. However, the main trigger responsible for the cascade of events resulting in local epidermal hyperplasia and recruitment of leukocytes in psoriatic lesions remains unidentified. It has been demonstrated that intra-epidermal infiltration of CD4<sup>+</sup> lymphocytes precedes the epidermal changes in developing psoriatic lesions [10]. Native and recombinant lymphokines can induce, in vivo and in vitro, the expression on keratinocytes of aberrant mole-

cules such as HLA-DR, gamma IP-10, and ICAM-1, which are normally expressed in psoriatic lesions [7-9]. Supernatants from unstimulated peripheral blood mononuclear cells (PBMC), Con-A stimulated PBMC, and allo-stimulated purified T lymphocytes were shown to be able to induce keratinocyte proliferation in vitro [11]. An important observation, clearance of severe psoriasis after allogeneic bone marrow transplantation, has recently been reported [12]. These studies indicate that the abnormalities in psoriatic keratinocytes may be triggered by, or are highly dependent on, bone-marrow-derived leukocytes and their cytokines. They also support the recent hypotheses on immune mechanisms in psoriasis [13-15].

Further evidence for the role of cellular immunity in psoriasis has been obtained in recent years. Immunophenotyping of the inflam-

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#### Abbreviations:

ACD: allergic contact dermatitis  
AET: 2-aminoethylisothiuroniumbromide  
APC: antigen-presenting cell(s)  
CD: cluster of differentiation  
CsA: cyclosporin A

EC: epidermal cell(s)  
FCS: fetal calf serum  
LC: Langerhans cell(s)  
Con-A: Concanavalin A  
MLR: mixed leukocyte reaction  
MECLR: mixed epidermal cell-leukocyte reaction  
MHC: major histocompatibility complex  
PBMC: peripheral blood mononuclear cells  
SRBC: sheep red blood cells  
 [<sup>3</sup>H]TdR: tritiated thymidine  
MoAb: monoclonal antibodies

matory infiltrate using cryostat sections showed a marked increase in the number of HLA-DR<sup>+</sup>/CD1a<sup>-</sup> dendritic cells and a slight increase in the number of HLA-DR<sup>+</sup>/CD1a<sup>+</sup> Langerhans cells in the epidermis from early psoriatic skin lesions [16,17]. The infiltrate in the dermis at this stage consisted mainly of activated (CD4<sup>+</sup>/CD25<sup>+</sup>/HLA-DR<sup>+</sup>/VLA $\alpha$ -4<sup>+</sup>) helper-inducer or memory T lymphocytes [16–18]. Sera of psoriasis patients also showed signs of T-cell activation. These sera were recently shown to contain elevated levels of soluble IL-2 receptor (TAC, [19]). Intra-epidermal CD4<sup>+</sup> T lymphocytes were observed in close apposition to HLA-DR<sup>+</sup> dendritic cells, a picture normally seen in contact hypersensitivity (DTH-like) skin reactions [14]. CD4<sup>+</sup> T lymphocytes appeared to be bound specifically to dermal capillary endothelia in psoriatic plaques, but not to those in uninvolved skin from either psoriasis patients or from normal individuals [20]. The presence of gamma interferon (IFN- $\gamma$ ) was demonstrated in suction blister fluid from psoriatic plaques, but not in blister fluid from uninvolved skin or in sera from psoriasis patients [21]. Gamma IFN-induced protein (gamma IP-10), like HLA-DR and ICAM-1, was expressed on keratinocytes and on the cells of dermal infiltrate in psoriatic plaques [22]. Because keratinocytes are not known to produce IFN- $\gamma$ , its most likely sources would be intra-epidermal or dermal T lymphocytes [23]. Although beneficial effects have also been reported, induction or exacerbation of psoriasis upon treatment with recombinant INF- $\gamma$  and INF- $\alpha$  have been well documented [24,25]. Cyclosporin A (CsA) was observed to be effective in clearing psoriasis [26–29]. The therapeutic effect of CsA and other therapeutic regimens in psoriasis was correlated to a decrease or disappearance of HLA-DR<sup>+</sup>/CD1a<sup>-</sup> cells in the epidermis, and to a decrease in CD4<sup>+</sup> T lymphocytes in the epidermal and dermal inflammatory infiltrate [17,18,30]. Considered together, these data suggest an involvement of CD4<sup>+</sup> T lymphocytes and HLA-DR<sup>+</sup>/CD1a<sup>-</sup> dendritic cells in the pathogenesis of psoriasis.

These observations have been corroborated by functional *in vitro* data. An increased *in vitro* autologous mixed epidermal cell–T lymphocyte reaction (MECLR), induced by unpurified epidermal cells, has recently been described in psoriasis patients [31–33]. In addition to an elevated autologous MECLR, Steinmüller et al also reported on the release of a leukocyte migration-inhibition factor by psoriatic EC, whereas cell-mediated cytotoxicity was not observed in psoriasis patients [32]. We have also observed an elevated *in vitro* autologous MECLR in psoriasis patients [34,35].

In the epidermis of normal skin, only one predominant type of APC, the MHC class II positive Fc and C3 receptor bearing Langerhans cell(s), has been observed [36,37]. Epidermal cell suspensions depleted of Langerhans cells are unable to present antigen to antigen-specific T lymphocytes [38]. Langerhans cells are also the main allostimulatory cells in the allogeneic MECLR [39,40]. Epidermal cell suspensions depleted of HLA-DR<sup>+</sup>/CD1a<sup>+</sup> Langerhans cells, therefore, do not stimulate allogeneic T cells [40,41]. The functional significance of the two dendritic cell subsets, namely HLA-DR<sup>+</sup>/CD1a<sup>+</sup> and HLA-DR<sup>+</sup>/CD1a<sup>-</sup> cells present in involved psoriatic skin, is not yet clear.

In this communication we report the results of *in vitro* studies on the kinetics of the autologous MECLR, the role of epidermal Langerhans cells and other APC subsets, and the role of MHC class II in T cell–APC interaction(s) in psoriasis.

## MATERIALS AND METHODS

**Patients and Controls** Twenty-four otherwise healthy patients (9 men, 15 women, median age 43) with active plaque-type, untreated psoriasis (needing clinical treatment) were tested. The autologous mixed leukocyte reaction (MLR) and the autologous MECLR were performed in all patients. Their PBMC were immunophenotyped using a broad panel of monoclonal antibodies (MoAb). As controls, autologous MLR and autologous MECLR were performed in 24 age- and sex-matched healthy volunteers (individuals without history or signs of skin disease undergoing abdominal plastic surgery) and in patients with active allergic contact dermatitis, mainly to nickel.

**Preparation of Epidermal Cell Suspensions** Epidermal sheets from involved and uninvolved skin of psoriasis patients were obtained using the suction blister technique [42]. Raising blisters in psoriatic plaques appeared quite difficult. The time necessary to obtain blisters was approximately 2.5–4 hours; even then, in some cases no blisters could be induced. Single cell suspensions of epidermal cells were prepared using standard methods [39]. Briefly, epidermal sheets were floated in a Ca<sup>++</sup>- and Mg<sup>++</sup>-free phosphate-buffered saline (PBS) solution containing 0.25% trypsin (ICN Biochemicals, Cleveland, OH) for 30 min, and gently agitated every 5 min, followed by a 15-min incubation in a 0.025% deoxyribonuclease (DNase; Sigma, St. Louis, MO) solution at room temperature. The epidermal cells were filtered through sterile 100- $\mu$ m and 30- $\mu$ m mesh nylon gauzes and resuspended in RPMI 1640 medium supplemented with penicillin, streptomycin, fresh L-glutamine, 20 mM Hepes (RPMI complete medium), 15% heat-inactivated human AB serum, and 0.01% DNase. Cells were counted using a hemocytometer and their viability was determined by trypan blue exclusion.

**Removal of Langerhans Cells and Other HLA-DR<sup>+</sup> Cells from Epidermal Cell Suspensions** Langerhans cells (HLA-DR<sup>+</sup>/CD1a<sup>+</sup>) or HLA-DR<sup>+</sup> cells were removed from the epidermal cell suspension by an immunomagnetic rosetting technique using anti-CD1a and anti-HLA-DR MoAb and goat anti-mouse-conjugated paramagnetic beads (Dynal, Oslo, Norway) [43]. Briefly, suspended epidermal cells were labeled for 30 min at 4°C with optimal dilutions of MoAb, washed 3 times, incubated with the conjugated magnetic beads, spun down, and the pellet incubated for 30 min. The pellet was carefully resuspended and the rosettes were counted using a hemocytometer. The rosetted fraction was concentrated to one side of the tube using a strong magnet. The non-rosetted cells were collected, leaving the rosetted cells adhered to the side of the tube. The latter procedure was repeated twice. In all depletion experiments, the numbers of CD1a<sup>+</sup> and HLA-DR<sup>+</sup> epidermal cells were determined using a two-step immunofluorescence technique. The number of rosetted cells was compared with the fluorescence data before depletion and the efficiency checked again later by immunofluorescent staining and counting under a microscope.

**Isolation of Peripheral Blood Mononuclear Cells** PBMC were isolated using density gradient centrifugation on Lymphoprep (density 1.077, Nyegaard, Oslo, Norway) [44]. All rinsing, also when isolating other cells, was done in RPMI complete medium containing 1% human AB serum.

**Isolation of Purified T Lymphocytes** T lymphocytes were isolated by a rosetting technique using 2-aminoethylisothiuroniumbromide (AET)-treated sheep red blood cells (SRBC) [45]. The purity of this fraction, as determined by immunofluorescence after incubation with anti-CD3(Leu-4) was approximately 95%, whereas 99% was CD2<sup>+</sup> (OKT11<sup>+</sup>). In this fraction, contamination with CD14<sup>+</sup> monocytes and CD20<sup>+</sup> B lymphocytes averaged 0–1% as determined by fluorescence microscopy or FACScan (Becton Dickinson, Sunnyvale, CA).

**Isolation and Purification of Peripheral Blood Monocytes** Monocytes were purified from the plastic adherent fraction of PBMC, or from the non-rosetting fraction after rosetting the T lymphocytes, using AET-treated SRBC as described by Gaudernack and Bjercke [46].

**Monoclonal Antibodies and Immunophenotyping of Cell Suspensions** The following MoAb were used: CD1a (66IIC7, Dr. M. van der Rijn, Amsterdam), CD2(OKT11, Ortho Diagnostic Systems, Raritan, NJ), CD3(Leu-4), CD4(Leu-3), CD8(Leu-2), CD16(Leu-11b), CD25(IL-2 receptor), HLA-DR (all from Becton Dickinson), CD20 (B1, Coulter Clone, Hialeah, FL), CD11b (OKM-1, Ortho Diagnostic Systems), transferrin receptor (T9, 66IG10, Dr. M. van der Rijn), CD14 (My3, Dr. J. D. Griffin, Boston, MA), and CD15 (VIM-D5, Dr. W. Knapp, Vienna, Aus-

tria). The MoAb and antisera used in blocking and panning experiments were dialyzed against complete RPMI medium without serum and stored at  $-20^{\circ}\text{C}$  until use. Immunophenotyping was performed using a two-step immunofluorescence technique as described by van Dongen et al [47]. Cells were counted using Standard Zeiss 16 microscopes equipped with a IV FL epi-illumination condenser (Carl Zeiss, Oberkochen, FRG). The percentage of positive cells was calculated by counting the number of cells showing positive fluorescence from a total of 200–400 cells, or by FACS analysis (Becton Dickinson).

**Autologous MECLR and MLR** Mixed cultures of unpurified epidermal cells and PBMC or purified T cells were set-up in quadruplicate in round-bottomed microtiter plates (Costar, Cambridge, MA). To each well,  $4 \times 10^4$  epidermal cells and  $1-2 \times 10^5$  PBMC or T cells were added in a final volume of 0.2 ml complete RPMI medium supplemented with 15% human AB serum. The autologous MLR comprised the spontaneous proliferation of separately plated fractions of PBMC and/or of cocultured purified T and autologous non-T cells (monocytes, dendritic cells, and B cells). In pilot experiments the stimulatory capacity of 30 Gy irradiated EC and PBMC were compared with that of unirradiated EC and PBMC. As the autologous response using unirradiated EC and PBMC did not differ significantly from that of 30 Gy-irradiated EC and PBMC, only unirradiated EC and PBMC were used in subsequent experiments. On days 6, 7, and 8, the cultures were pulsed with  $0.5 \mu\text{Ci} = 18.5 \text{ kBq}$  tritiated thymidine ( $[^3\text{H}]\text{TdR}$ , 185 GBq/mmol, Amersham, Buckinghamshire, UK) and harvested 8 h later using a semi-automatic cell harvester (Skatron, Oslo, Norway) onto glass fiber filters. The amount of  $[^3\text{H}]\text{TdR}$  that was incorporated was determined by liquid scintillation counting (Tri-Carb 1500, Packard Instrument Co., Downersgrove, IL). The results were expressed as mean  $\pm$  SEM disintegrations per min (dpm) of quadruplicate cultures.

Cell suspensions were fixed with 0.01% glutaraldehyde (Grade I, Sigma Chemical Co.) for 30 sec on ice, followed by five rinses with cold complete RPMI medium containing 15% human AB serum.

**Blocking of the Autologous MECLR with MoAb** In some experiments MoAb specific for monomorphic MHC class II (anti-HLA-DR, Becton and Dickinson) were added to the autologous MECLR cultures. In other studies at our laboratory this MoAb effectively blocked MHC class II restricted responses.

**Preincubation of EC with Cyclosporin A** The effect of Cyclosporin A (CsA) on the autologous MECLR was studied by incubating EC from psoriatic lesions for 1 h at  $37^{\circ}\text{C}$  with  $2.5 \mu\text{g}/\text{ml}$  CsA (pure substance, provided by Sandoz, Basel, Switzerland) in complete medium containing 1% human AB serum. After incubating the EC were rinsed five times with warm ( $37^{\circ}\text{C}$ ) complete medium containing 15% human AB-serum. The last two rinses did

not contain detectable levels of CsA as measured using the standard Sandoz Cyclosporin A radioimmunoassay (CsA-RIA) kit.

**Statistical Analysis** The results were analyzed with STATA (computer program for statistical analysis, Computing Resource Center, Los Angeles, CA). The test used to determine statistical significance is mentioned together with the p values in abbreviated form, e.g., the Wilcoxon test (WT), Wilcoxon signed rank sum test (WSRT).

## RESULTS

**The Autologous MECLR in Psoriasis Patients and in Controls** The PMBC of all psoriasis patients showed a clear proliferative response to autologous unpurified epidermal cells (Table I). The level of the responses did not correlate with the clinical severity of their psoriasis and showed considerable inter-patient variability. Unpurified epidermal cells from psoriatic lesions induced higher proliferation rates in autologous T lymphocytes than unpurified epidermal cells from uninvolved skin ( $p < 0.001$ , WT). The autologous MLR was normal in psoriasis patients (Table I). Gradual increment in the number of epidermal cells from psoriasis lesions resulted in increased proliferative responses of PBMC, showing maximum T lymphocyte proliferation at  $4-8 \times 10^4$  epidermal cells per well (Fig 1). To exclude the possibility that the observed proliferation in the autologous MECLR in psoriasis was due to the presence of epidermal T lymphocytes that were being stimulated and expanded in vitro, the EC were gamma irradiated (30 Gy) in some experiments. The results of these experiments are shown in Table II. It can be seen that the differences between irradiated and non-irradiated EC were not significant (WSRT).

In the age- and sex-matched control group of healthy volunteers and in patients with allergic contact dermatitis, no significant proliferation was observed in the autologous MECLR, using  $4 \times 10^4$  stimulator EC and  $10^5$  responder PBMC (Table I).

The immunophenotypic pattern of PBMC samples from psoriasis patients did not differ significantly from those of healthy controls and patients with active allergic contact dermatitis (Table III). The only exception was that most PBMC samples from psoriasis patients contained significantly more transferrin receptor ( $4.3\%$ ;  $\text{CD}71^+$ ) and IL-2 receptor ( $4.4\%$ ;  $\text{CD}25^+$ ) positive activated T lymphocytes than transferrin receptor ( $0.5\%$ ;  $\text{CD}71^+$ ) and IL-2 receptor ( $0.2\%$ ;  $\text{CD}25^+$ ) positive activated T lymphocytes in PBMC samples from healthy controls ( $p < 0.001$ , WT; Table III). These results may be influenced by the fact that PBMC fractions from three patients contained high numbers (mean 17%) of activated T lymphocytes. If these three patients were excluded, the mean percentage of  $\text{CD}25^+$  and  $\text{CD}71^+$  cells in psoriasis patients was 2.9%. Epidermal cells from psoriatic lesions contained more HLA-DR $^+$  cells than uninvolved and control skin ( $p < 0.0005$ , WT, Table IV).

**Table I.** Proliferation Rates (mean  $\pm$  SEM) in the Autologous MECLR and in Different Cell Subsets from Psoriasis Patients, Healthy Controls, and Allergic Contact Dermatitis (ACD) Patients<sup>a</sup>

	EC	T cells	PBMC <sup>b</sup>	EC + PBMC <sup>c</sup>
Psoriasis P <sup>d</sup>	94 $\pm$ 6.4 (n = 16)	789 $\pm$ 132 (n = 16)	2177 $\pm$ 455 (n = 24)	42,688 $\pm$ 6730 (n = 24)
Psoriasis N <sup>d</sup>	81 $\pm$ 7.3 (n = 8)	579 $\pm$ 140 (n = 8)	1987 $\pm$ 634 (n = 8)	20,369 $\pm$ 4320 <sup>e</sup> (n = 8)
Controls	89 $\pm$ 7.1 (n = 16)	621 $\pm$ 118 (n = 16)	1907 $\pm$ 373 (n = 24)	2178 $\pm$ 320 (n = 24)
ACD patients	76 $\pm$ 6.9 (n = 16)	797 $\pm$ 214 (n = 16)	1746 $\pm$ 420 (n = 16)	1957 $\pm$ 401 (n = 16)

<sup>a</sup> Unpurified epidermal cells (EC) ( $4 \times 10^4$  EC/well), purified T cells ( $10^5$  cells/well), and PBMC ( $10^5$  cells/well) were cultured separately.

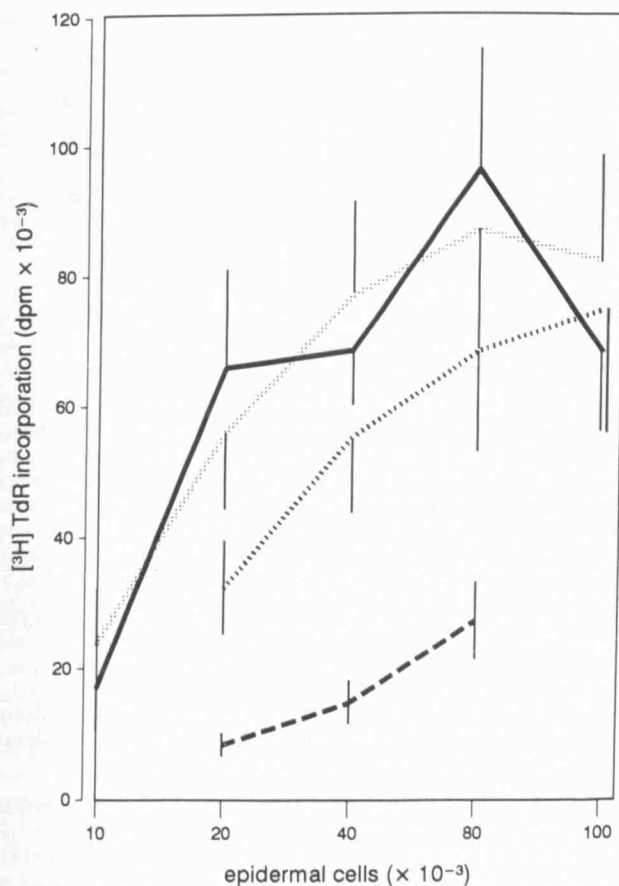
<sup>b</sup> The autologous MLR is represented here. PBMC were plated in two separate fractions (total  $10^5$  well), in this case using unirradiated stimulator PBMC. The results are shown as the mean  $\pm$  SEM dpm of the maximum day- $[^3\text{H}]\text{TdR}$  incorporation.

<sup>c</sup> In the autologous MECLR  $4 \times 10^4$  unirradiated EC were cocultured for 6–8 d with  $10^5$  autologous PBMC.

<sup>d</sup> Psoriasis P = EC from psoriasis plaque; Psoriasis N = EC from uninvolved skin from psoriasis patients.

<sup>e</sup>  $p < 0.001$ , WT, when compared with MECLR using EC from psoriasis plaques.





**Figure 1.** Dose-response curves of the autologous MECLR. Increasing numbers of unpurified epidermal cells from psoriasis lesions were cocultured with fixed numbers of autologous PBMC ( $10^5$  cells/well). On days 6, 7, and 8 the cultures were pulsed with  $0.5 \mu\text{Ci}$  ( $= 18.5 \text{ kBq}$ )  $[^3\text{H}]\text{TdR}$  per well and harvested 8 h later. The results are the arithmetic mean  $\pm$  SEM dpm of quadruplicate cultures on the day of maximum proliferation. Curve, the result of an individual patient.

**The Roles of Skin APC and Peripheral Blood APC in the Autologous MECLR** When purified T lymphocytes from psoriasis patients were cocultured with autologous unpurified EC, lower proliferation rates than those after coculturing unpurified PBMC with EC were observed (Table V). When the non-T cell fraction (after AET rosetting, APC in Table V) was cocultured with autologous EC no significant proliferation occurred (Table V). This dem-

**Table III.** Immunophenotyping of PBMC Samples from Psoriasis Patients, Controls, and Allergic Contact Dermatitis (ACD) Patients<sup>a</sup>

MoAB	Psoriasis (n = 24)	Controls (n = 60)	ACD (n = 24)
CD20	$6.1 \pm 2.5$	$7.0 \pm 3.7$	$6.2 \pm 3.6$
CD3	$57.7 \pm 8.2$	$59.1 \pm 13.1$	$61.1 \pm 9.6$
CD4	$38.2 \pm 6.6$	$38.4 \pm 10.9$	$38.1 \pm 9.5$
CD8	$20.5 \pm 6.3$	$20.8 \pm 5.4$	$23.1 \pm 5.4$
CD4/CD8	$2.0 \pm 0.9$	$2.0 \pm 0.8$	$1.7 \pm 0.8$
CD14	$22.6 \pm 8.6$	$18.9 \pm 8.5$	$19.1 \pm 7.7$
HLA-DR	$29.3 \pm 8.3$	$27.5 \pm 8.8$	$27.6 \pm 8.1$
CD25(IL-2R)	$4.4 \pm 9.7^b$	$0.2 \pm 0.2$	$1.1 \pm 0.6$
CD71(TFR)	$4.3 \pm 4.8^b$	$0.5 \pm 0.5$	$1.7 \pm 1.2$

<sup>a</sup> The percentage mean  $\pm$  SD of monocyte and lymphocyte subsets in PBMC samples from psoriasis patients, healthy controls and ACD patients. Clusters of differentiation (CD) are shown.

<sup>b</sup>  $p < 0.001$ , WT, when compared to the results in healthy controls.

<sup>c</sup> TFR = transferrin receptor.

onstrated that T lymphocytes were proliferating in the autologous MECLR. Proliferation of T lymphocytes via back stimulation in the EC suspension was excluded, because irradiation of the EC suspension did not affect the proliferative response in the autologous MECLR (Table III).

The role of peripheral blood and skin APC was studied by eliminating their antigen-processing function by fixing with 0.01% glutaraldehyde for 30 sec. Addition of fixed peripheral blood APC to EC and to purified T lymphocytes resulted in a significantly lower response than those using unfixed peripheral blood APC ( $p < 0.02$ , WSRT). However, when the unpurified fixed EC suspension was used, proliferation was almost completely inhibited, even in the presence of unfixed peripheral blood APC ( $p < 0.02$ , WSRT) (Table V). These results suggest that peripheral blood APC have an accessory rather than an initiating function in the autologous MECLR.

**The Role of HLA-DR<sup>+</sup>/CD1a<sup>+</sup> and HLA-DR<sup>+</sup>/CD1a<sup>-</sup> Epidermal APC in the Autologous MECLR** To determine which epidermal APC subset was involved in the activation of T lymphocytes in psoriasis, EC were depleted of HLA-DR<sup>+</sup>/CD1a<sup>+</sup> cells only, or of both HLA-DR<sup>+</sup>/CD1a<sup>+</sup> and HLA-DR<sup>+</sup>/CD1a<sup>-</sup> cells. When the CD1a-depleted EC fraction from psoriasis patients was cocultured with autologous T lymphocytes, a minimal inhibition of the MECLR was observed. In Fig 2, the paired results of four representative patients are shown. In the remaining patients paired, studies could not be performed owing to insufficient number of EC. However, individual depletion and HLA-DR blocking studies were performed. The results of these studies were in agreement with those shown in Fig 2. Removal of all HLA-DR<sup>+</sup> cells from the EC suspension from psoriasis patients resulted in a strong inhibition (mean 80–90%) of the autologous MECLR. The same inhibition was observed by the addition of anti-monomorphic HLA-DR MoAb to these cultures (Fig 2). These results indicate that epider-

**Table II.** The Effect of Irradiated Stimulator EC on the Autologous MECLR in Psoriasis Patients<sup>a</sup>

Patient (n = 8)	EC + PBMC <sup>b</sup>	EC/irr + PBMC	Autologous MLR <sup>c</sup>
1	$27,801 \pm 4,885$	$24,183 \pm 3,619$	$2,135 \pm 1,068$
2	$22,808 \pm 5,000$	$20,858 \pm 1,930$	$2,858 \pm 667$
3	$18,145 \pm 4,202$	$20,338 \pm 3,537$	$795 \pm 317$
4	$17,102 \pm 2,386$	$22,856 \pm 4,637$	$1,403 \pm 216$
5	$43,041 \pm 3,009$	$38,626 \pm 2,156$	$3,566 \pm 691$
6	$22,052 \pm 3,061$	$22,175 \pm 3,551$	$2,405 \pm 1,251$
7	$20,524 \pm 1,661$	$20,554 \pm 1,973$	$1,643 \pm 341$
8	$16,184 \pm 3,286$	$15,469 \pm 724$	$1,224 \pm 356$

<sup>a</sup> The results are shown as the mean  $\pm$  SEM cpm of the maximum  $[^3\text{H}]\text{TdR}$  incorporation.

<sup>b</sup> In the autologous MECLR,  $4 \times 10^4$  irradiated and unirradiated EC from psoriasis plaques were cocultured for 6–8 d with  $10^5$  autologous PBMC. The results obtained using irradiated psoriatic EC are not significantly different from those obtained using unirradiated EC (WSRT).

<sup>c</sup> In the autologous MLR  $10^5$  PBMC were co-cultured with  $10^5$  irradiated autologous PBMC.

**Table IV.** The Mean  $\pm$  SD Percentage of HLA-DR<sup>+</sup> and CD1a<sup>+</sup> Cells in EC Suspensions from Psoriasis Patients and Controls

	HLA-DR <sup>+</sup>	CD1a <sup>+</sup>
Psoriasis P <sup>a</sup> (n = 10)	$5.1\% \pm 1.6^b$	$1.8\% \pm 0.7$
Psoriasis N <sup>a</sup> (n = 8)	$1.9\% \pm 0.6$	$1.6\% \pm 0.4$
Controls (n = 10)	$1.6\% \pm 0.4$	$1.5\% \pm 0.4$

<sup>a</sup> Psoriasis P = EC from psoriasis plaques; Psoriasis N = EC from uninvolved skin of psoriasis patients.

<sup>b</sup> Significance  $p < 0.0005$ , WT, when compared to the results in healthy controls.

**Table V.** The Proliferative Activity (mean  $\pm$  SEM) of In Vitro (Co)cultured Peripheral Blood Cell Subsets and EC from Psoriasis Patients

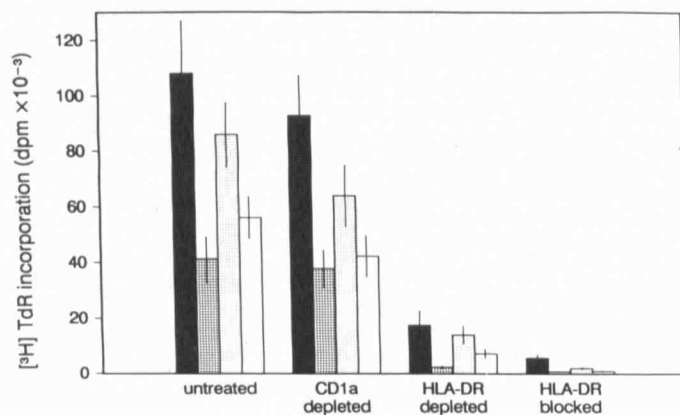
Cocultured Cell Types	Proliferation Rate, Mean $\pm$ SEM in dpm <sup>a</sup> (n = 8)
Basal Proliferation Rates	
EC	127 $\pm$ 35
PBMC	2,371 $\pm$ 794
T cells	722 $\pm$ 102
APC	559 $\pm$ 173
EC + APC	831 $\pm$ 171
T + APC	1,107 $\pm$ 237
The Role(s) of Peripheral Blood and Epidermal APC in the Autologous MECLR <sup>b</sup>	
EC + PBMC	54,024 $\pm$ 9,730
EC + T	35,283 $\pm$ 13,508
EC + T + APC	61,610 $\pm$ 16,690
EC + T + APCfix	32,125 $\pm$ 10,819
ECfix + T + APC	1,869 $\pm$ 345

<sup>a</sup> Proliferation rates represent the mean  $\pm$  SEM dpm [<sup>3</sup>H]TdR incorporation values of quadruplicate cultures of eight patients.

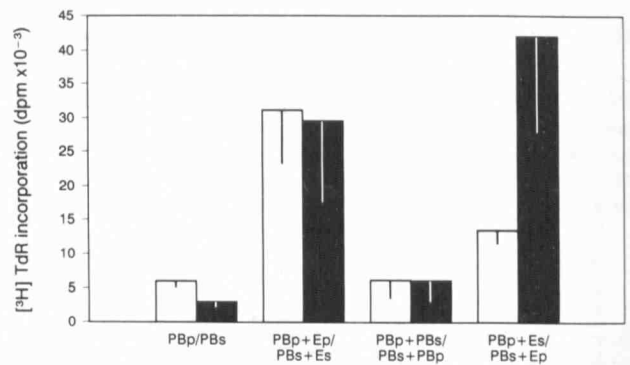
<sup>b</sup> Unpurified EC ( $4 \times 10^4$  EC/well) from psoriasis lesions were cocultured with autologous PBMC, purified peripheral blood T cells ( $10^5$  cells/well), or with autologous T cells and non-T cells (APC) ( $4 \times 10^4$  cells/well). Glutaraldehyde (0.01%) fixed APC or EC were also cocultured with purified T cells and unfixed EC or APC. On days 6, 7, and 8 the cultures were pulsed with 0.5  $\mu$ Ci (= 18.5 kBq) [<sup>3</sup>H]TdR per well and harvested 8 h later.

mal HLA-DR<sup>+</sup>/CD1a<sup>+</sup>-APC mainly stimulate autologous T lymphocytes in the MECLR, whereas HLA-DR<sup>+</sup>/CD1a<sup>+</sup> APC did so weakly or not at all.

Our observation that EC from uninvolved psoriatic skin stimulated autologous T lymphocytes raised the question of whether EC from normally appearing skin from psoriasis patients would be able to stimulate in the autologous MECLR. This was studied using PBMC and irradiated EC from an identical twin discordant for psoriasis (women, aged 67, the affected sibling having psoriasis for over 50 years). It appeared that EC from apparently normal skin from the unaffected sibling was able to stimulate autologous PBMC and PBMC from the affected sibling and vice versa (Fig 3). Although these results were obtained from a single set of twins, they



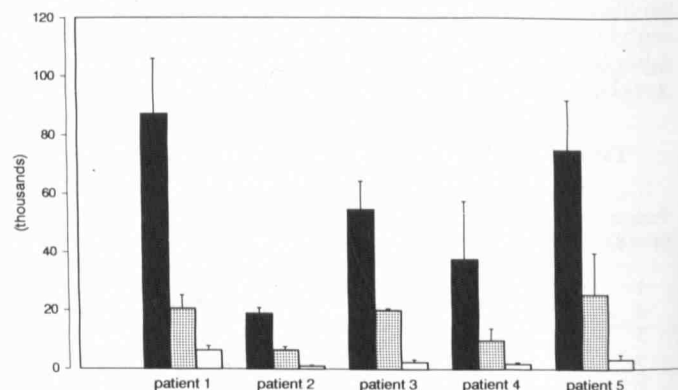
**Figure 2.** The role of MHC class II positive skin APC subsets in the autologous MECLR. Purified T lymphocytes ( $10^5$  cells/well) were co-cultured with autologous untreated EC, CD1a depleted EC, or CD1a plus HLA-DR-depleted EC, all at a concentration of  $4 \times 10^4$  EC/well. On days 6, 7, and 8 the cultures were pulsed with 0.5  $\mu$ Ci (= 18.5 kBq) [<sup>3</sup>H]TdR per well and harvested 8 h later. Blocking was performed by addition of dialyzed anti-HLA-DR MoAb (Becton Dickinson, dilution 1:100) to the cultures. Filled, bold speckled, fine speckled, and blank bars each represents the arithmetic mean  $\pm$  SEM dpm of quadruplicate cultures on the day of maximum proliferation of an individual patient. Paired results of four representative patients are shown.



**Figure 3.** Comparison of the abilities of EC and PBMC from an identical twin discordant for psoriasis to stimulate in a two-way autologous MECLR. Blank bars, the proliferation obtained with responder cells from the psoriasis patient (PBp); solid bars, the proliferation obtained with responder cells from the unaffected twin sister (PB). Irradiated unpurified EC (from uninvolved skin) and PBMC were cocultured with fixed numbers of autologous responder PBMC ( $10^5$  cells/well) from the psoriasis patient and unaffected sister and vice versa. On days 6, 7, and 8 the cultures were pulsed with 0.5  $\mu$ Ci (= 18.5 kBq) [<sup>3</sup>H]TdR per well and harvested 8 h later. The results are the arithmetic mean  $\pm$  SEM dpm of quadruplicate cultures on the day of maximum proliferation.

indicated that normal skin from a genetically predisposed individual without the disease had a similar capacity to stimulate proliferation in the MECLR as in uninvolved psoriatic skin.

**Inhibition of the Autologous MECLR by CsA** Preincubation of EC with 2.5  $\mu$ g CsA per ml resulted in a clear inhibition of the autologous MECLR (n = 5, mean = 69%  $\pm$  7% SEM, p < 0.05, WSRT) (Fig 4). Pretreatment with the solvent (ethanol/PBS 1:1000) alone had no effect on the accessory capacity of EC or PBMC (data not shown). To check for the possible release of CsA from the preincubated EC, the CsA levels in EC culture supernatants were measured. On days 7 and 8 of culture, low levels i.e., 25 ng CsA per ml, could be measured in three of five culture supernatants. These levels appeared not to interfere with the assay, in that addition of up to 75 ng/ml CsA did not inhibit the autologous MECLR (Table VI) or the Con-A response (data not shown). Others have shown that when free CsA was added at the beginning of a MECLR, significant inhibition occurred at concentrations in



**Figure 4.** The effect of preincubation of lesional EC with 2.5  $\mu$ g CsA per ml on the autologous MECLR. Lesional EC were incubated with CsA in RPMI complete medium containing 1% human AB serum for 1 h at 37°C. The cells were washed 5 times with warm RPMI complete medium containing 15% human AB serum. On days 6, 7, and 8 the cultures were pulsed with 0.5  $\mu$ Ci (= 18.5 kBq) [<sup>3</sup>H]TdR per well and harvested 8 h later. The results are the arithmetic mean  $\pm$  SEM dpm of quadruplicate cultures on the day of maximum proliferation. The difference between the treated (speckled bars) and untreated (solid bars) is statistically significant (p < 0.05, WSRT). Blank bars, the autologous MLR of each patient.

**Table VI.** The Effect of CsA Pretreated EC on the Autologous MECLR and Neutralization of the Inhibitory Effect by the Addition of Fresh EC<sup>a</sup>

	AMECLR	AMECLR/CsA Pulse	AMECLR/CsA Pulse + Fresh EC
75 ng CsA	18,348 ± 3,632 <sup>a</sup> 19,882 ± 3,214	7,828 ± 4,885 <sup>a</sup> nt	21,628 ± 9,503 <sup>b</sup> nt

<sup>a</sup> Unpurified EC ( $4 \times 10^4$  EC/well) from psoriasis lesions were co-cultured with autologous PBMC ( $10^5$  cells/well). On days 6, 7, and 8 the cultures were pulsed with 0.5  $\mu$ Ci (= 18.5 kBq) [<sup>3</sup>H]TdR per well and harvested 8 h later.

<sup>b</sup> Proliferation rates represent the mean  $\pm$  SEM cpm [<sup>3</sup>H]TdR incorporation of quadruplicate cultures in 5 patients.

the 100 ng/ml range [48]. In addition, when EC preincubated with CsA and untreated EC were cocultured together in equal numbers ( $4 \times 10^4$  each), the CsA-induced inhibition of proliferation in the autologous MECLR was neutralized (Table VI). This suggests that the observed inhibitory effect was caused by the effect of CsA on the accessory function of EC and not by direct inhibition of the proliferation of the co-cultured lymphocytes.

### DISCUSSION

The results indicate that in psoriasis patients autologous unpurified EC stimulate T-lymphocyte proliferation. Such a reaction could not be demonstrated in the control group of healthy volunteers and in patients with allergic contact dermatitis. Elevated proliferation has been reported in the autologous MECLR in a limited number of healthy individuals [49]. The latter observation cannot be compared to ours, because the number of Langerhans cells used differed considerably, namely  $25 \times 10^3$  purified LC per well versus approximately 500–2000 LC in our experiments. In the autologous MLR, generally high cell numbers are used (up to  $5 \times 10^5$  per well) [50,51]. In our study, the number of stimulator EC ( $2-4 \times 10^4$  per well) were equal to the number of EC used to present antigens such as nickel or tetanus. Using the latter system, low background and high antigen-specific proliferation values are obtained. In the present studies, proliferation in the autologous MECLR was a consistent finding in psoriasis patients, whereas, using identical in vitro conditions, it has not yet been reported in a large number of healthy individuals (personal observations) [52,53]. In cutaneous T-cell lymphoma, the autologous MECLR was increased when EC from involved skin were used. However, low proliferation values (background) were observed when EC from uninvolved skin were used as stimulators [52].

To date, elevated autologous MECLR have been reported in psoriasis and cutaneous T-cell lymphoma [31,32,52]. Recently, it was reported that also after four MED UVB irradiation autologous MECLR activity could be demonstrated in which suppressor-inducer and suppressor/cytotoxic T lymphocytes were preferentially activated by HLA-DR<sup>+</sup>/CD1a<sup>-</sup> epidermal APC [53]. In addition to an elevated autologous MECLR, EC from psoriasis lesions were found to produce leukocyte migration-inhibition factor, whereas these features were not observed in lichen planus [32]. The latter finding seems surprising at first, because lichen planus is known to exhibit some (immuno)histologic characteristics of psoriasis, i.e., a prominent mononuclear inflammatory infiltrate and HLA-DR<sup>+</sup>/CD1a<sup>-</sup> epidermal cells. These diseases, however, also have fundamental clinical and (immuno)histologic differences. Thus, it is conceivable that both diseases evolve via different pathophysiologic pathways. Based on clinical and experimental observations, it was recently reported whether these two diseases were mutually exclusive [54].

The proliferative response in the autologous MECLR in psoriasis patients depends on the number of unpurified epidermal cells added to the cultures, but is inducible even by relatively small numbers of autologous EC ( $10^4$  EC, containing  $\pm 500$  HLA-DR<sup>+</sup> cells/well). Although it has been shown that psoriatic epidermis contains considerable number of T lymphocytes, the contribution of these cells to the proliferative response via backstimulation seems negligible (Tables II and V). We calculated an average of one T cell per  $10^4$  EC using a limiting dilution procedure. This would correspond to four T cells per well (data not shown). Even under optimal culture con-

ditions, such low numbers of T cells would not contribute significantly to the observed high incorporation of [<sup>3</sup>H]Thymidine. The results obtained using gamma-irradiated EC exclude the possibility that the proliferation in the autologous MECLR was due to the presence of epidermal T lymphocytes that were being backstimulated and expanded in vitro. However, it may well be that this observation does not strictly reflect the in vivo situation. The method used to isolate EC (e.g., trypsin/DNase digestion, mechanical disruption, and sieving) may be responsible for the observed inability of lesional T lymphocytes to participate in vitro in the autologous MECLR. The stimulation observed using EC from uninvolved skin also argues against an important role for lesional T lymphocytes in the autologous MECLR in psoriasis, because activated T cells are hardly observed in uninvolved skin. Uninvolved skin of psoriatic patients has been shown to contain small numbers of HLA-DR<sup>+</sup>/CD1a<sup>-</sup> APC (Table III and reference [48]). The autologous MECLR induced by EC from uninvolved skin could be blocked by addition of anti-HLA-DR MoAb or by depletion of HLA-DR<sup>+</sup> cells from the EC suspensions (data not shown). The presence of these HLA-DR<sup>+</sup>/CD1a<sup>-</sup> epidermal APC, in combination with metabolically and biochemically altered keratinocytes, could explain the in vitro elicitation of an autologous MECLR by EC from uninvolved skin.

Unpurified EC from psoriasis patients stimulate autologous T lymphocytes in the autologous MECLR and therefore contain the necessary APC to initiate this reaction. The results of the dose-response studies, and depletion and fixation experiments indicate that HLA-DR<sup>+</sup>/CD1a<sup>-</sup> APC are the principal stimulators in the EC suspensions from psoriatic lesions. The proliferation observed in this system was induced only by autologous unpurified EC and not by autologous peripheral blood APC. Although it is still unclear whether the autologous MLR is exclusively antigen driven, it is clear that MHC class II plays a central role [50]. It may be that epidermal HLA-DR<sup>+</sup> APC present an antigen (putative psoriasis related) to these lymphocytes [13]. The putative antigen is possibly MHC class II associated with X, where X represents a yet-undefined (probably skin-specific) peptide residing in the peptide-binding groove of the MHC class II molecule [33,50,53,55].

The real specificity of in vitro cloned putative autoreactive T cells remains of primary concern [56]. Non-specific effects induced as a result of cross-reactivity especially with xenogeneic proteins (e.g., FCS components, SRBC components) in the culture medium should be minimized or prevented [56]. The chance of such effects in short term in vitro memory-T cell responses as performed by us is negligible, because we used medium containing human AB serum for culturing and rinsing. The occurrence of such an interference in our system is also highly unlikely, because whole PBMC in the autologous MLR, cocultured purified T and non-T cells from psoriasis patients, and cocultured PBMC and EC in controls, showed only background levels of proliferation (Tables I and V). The results of fixation studies suggest that glutaraldehyde fixation inhibited the re-expression of such an antigen in association with MHC class II on the plasma membrane of EC. This possibility cannot be ruled out, because EC were fixed immediately after trypsinization. This meant that the antigen is probably trypsin sensitive and that it is re-expressed on the membrane of EC in the initial phase of culturing. Induction of the autologous MECLR by sheer increased numbers of (bare) HLA-DR molecules occurring in psoriasis lesions is unlikely, as HLA-DR molecules are not affected by trypsin and/or glutaral-



dehyde treatment. This was confirmed in immunofluorescence studies in which no alteration in HLA-DR expression on PBMC and EC was observed following trypsin and/or glutaraldehyde treatment. Removal of 40 to 90% of the total HLA-DR<sup>+</sup> EC population by depleting HLA-DR<sup>+</sup>/CD1a<sup>+</sup> LC from the EC resulted only in mild inhibition of the autologous MECLR (Fig 3). Furthermore, fixation with glutaraldehyde did not inhibit the antigen-presenting function of nickel-pulsed EC (data not shown), indicating that at the concentrations of glutaraldehyde used, HLA-DR molecules remained functional. These results suggest that the stimulatory capacity of epidermal APC is altered in psoriatic lesions. Indeed, epidermal APC from psoriatic lesions have recently been shown to stimulate allogeneic T lymphocytes more strongly than EC from uninvolved psoriatic skin or from healthy individuals [33]. This increased allogeneic stimulation was attributed to an increased number of HLA-DR<sup>+</sup>/CD1a<sup>+</sup> cells in lesional EC suspensions. In this study, we also observed higher numbers of HLA-DR<sup>+</sup> cells than CD1a<sup>+</sup> cells in EC suspensions from psoriatic lesions ( $p < 0.0005$ , WT; Table IV). We analyzed the role of these two (dendritic) cell subsets in the autologous MECLR by selective depletion of one or both of these APC from the EC suspensions. Our observation that HLA-DR<sup>+</sup>/CD1a<sup>+</sup> LC were poor stimulators of the autologous MECLR is in sharp contrast to the superior capacity of these cells to present native antigen to specific T lymphocytes or T cell hybridomas [33,57–60]. In the latter system, depletion of HLA-DR<sup>+</sup>/CD1a<sup>+</sup> cells from fresh EC suspensions resulted in a complete inhibition of proliferation. This discrepancy is attributed to the findings that in normal human epidermis the LC is the only population that expresses HLA-DR, whereas in psoriatic lesions other cell types also express HLA-DR [16–18,41]. The current results show that a HLA-DR<sup>+</sup>/CD1a<sup>+</sup> APC subset indeed plays a crucial role in the autologous MECLR.

The inhibition of the autologous MECLR by CsA is not surprising, as it has been shown that CsA is able to inhibit the antigen-presenting function of APC in other systems [61,62]. It was recently demonstrated that the antigen-presenting capacity in the skin of psoriasis patients undergoing oral CsA therapy was significantly inhibited in an allogeneic MECLR model [48]. Here we show that in vitro treatment of psoriatic EC with CsA also inhibited their capacity to stimulate autologous T lymphocytes. The latter results support the conclusions by Cooper et al that one of the modes of action of CsA in psoriasis occurs via the inhibition of antigen-presenting activity of lesional epidermal APC [48].

Recently, it was reported that cultured LC lost the expression of CD1 and cytoplasmic Birbeck granules, while concurrently the expression of MHC class II was upregulated and powerful accessory functions were acquired [57–60,63]. Cultured LC became potent stimulators of both autologous and allogeneic MECLR [57–60,63]. The data on the capacity of fresh and cultured LC to present and process antigen remain controversial [60,63 vs 59]. Streilein et al attempted to clarify this matter by using murine cells, and showed that fresh LC processed and presented native antigen more efficiently than cultured LC, whereas cultured LC appeared equally efficient in presenting preprocessed antigen and superior at stimulating allogeneic T lymphocytes [63]. In the same study [63], it was also shown that cultured LC exhibit a unique feature that was not shared by fresh LC, namely an extraordinary capacity to stimulate syngeneic T lymphocytes. The authors considered "fresh and cultured LC as the in vitro representatives, respectively, of their in vivo counterparts: intraepidermal LC, and LC that have migrated to draining lymph nodes (nodal LC)."

Elevated levels of cytokines and of inflammatory mediators may alter the microenvironment in psoriatic lesions in such a way that in vivo lymph node or in vitro culture conditions are simulated. Because the phenotype and some functional characteristics of "cultured" LC and HLA-DR<sup>+</sup>/CD1a<sup>+</sup> APC occurring in psoriasis lesions may be similar, it is tempting to speculate that this subset resembles the in vivo equivalent of the in vitro "cultured" LC. It remains to be established whether the HLA-DR<sup>+</sup>/CD1a<sup>+</sup> APC subset in EC in other dermatoses exhibit similar functional characteristics.

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